

CLAIMS

1. An isolated protein comprising at least a subsequence of the amino acid sequence of LTA<sub>4</sub> hydrolase, which exhibits a three-dimensional form essentially as disclosed in Table 9 by the parameters defining atom 1 to atom 4876, said subsequence being capable of participating in the control of the an enzymatic pathway, such as the leukotriene cascade, or a functionally equivalent part, derivative or conformational analogue thereof.
2. A protein according to claim 1, which comprises an enzymatically active site defined in the following table:

	Left wall	Right wall
1		Lys608, Asp606, Lys605, Lys354, Thr355
2	Phe356, Phe362	Gln544, Asp573, Lys572, Arg568
3	Val376	Lys565, Arg540, Leu507
4	Ser380, Ser352, Glu348	Pro569
5	Tyr378, Glu348	Arg563, Glu533, Phe536, Arg537, Tyr267
6	Tyr383, Phe314, Glu318, Glu384, Arg326	
7	Gly268, Gly269, Met270	His295, Asn341, Phe340
8	Ser288, His497	Glu325, Asn291

3. A protein according to claim 2, which is an enzyme having a metallohydrolase activity capable of participating in the regulation of enzyme activities in biochemical pathways, wherein said enzymes have structures similar to the ones defined in claim 2.
4. A protein according to claim 1, which comprises an enzymatically active site defined by the following amino acids: Gln136; Ala137; Tyr267; Gly268; Gly269; Met270; Glu271; Val292; His295; Glu296; His299; Glu318; Tyr378; Tyr383; Arg563; Lys565.
5. A protein according to claim 1, which comprises an enzymatically active site defined by the following amino acids: Gln136; Ala137; Tyr267; Gly268; Gly269; Met270; Glu271; Val292; His295; Glu296; His299; Trp315; Glu318; Val322;

Phe362; Val367; Leu369; Pro374; Asp375; Ile372; Ala377; Pro382; Tyr378;  
Tyr383; Arg563; Lys565.

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6. A compound which is substantially complementary to a protein according to any one of claims 1-5.

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7. A compound according to claim 6, which is substantially complementary to an enzymatically active site of said protein and which is capable of specifically inhibiting said enzymatic activity.

8. A compound according to claim 7, which is an inhibitor of a metallohydrolase enzyme.

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9. An isolated complex, which is comprised of a protein according to claim 1-5 and a complementary compound according to any one of claims 6-8, wherein the three-dimensional structure of LTA<sub>4</sub> hydrolase is essentially as disclosed in Table 9 by the parameters defining atom 1- atom 4876, or a functionally equivalent part, derivative or conformational analogue of such a complex.

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10. A complex according to claim 9, wherein the protein complexed with LTA<sub>4</sub> hydrolase is selected from the group which consists of bestatin, thiolamine or hydroxamic acid, or a functionally equivalent part, derivative or conformational analogue of such a complex.

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11. Use of the parameters of a protein according to any one of claims 1-5, a compound according to any one of claims 6-8 or a complex according to claim 9 or 10 in drug design, such as in molecular modeling, direct structure-based design and/or combinatorial chemistry.

12. Use according to claim 11, wherein said parameters are selected from the parameters disclosed in Table 9 defining atom 1- atom 4876.

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13. Use according to claim 11 or 12, wherein said drug is for the treatment and/or prevention of disorders involving acute and chronic inflammatory and/or allergic symptoms, said disorder being selected from the group consisting of arthritis, inflammatory bowel disease (IBD), psoriasis, chronic obstructive pulmonary disease (COPD), and acquired immune deficiency syndrome (AIDS).

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14. Use according to claim 11 or 12, wherein said drug is for the treatment and/or prevention of proliferative disorders, such as neoplasias and/or cancer.

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15. Use according to claim 11 or 12, wherein said drug is for the treatment and/or prevention of disorders caused by the lethal factor of *Bacillus anthracis*, e.g. anthrax.
- 5 16. A method for screening LTA<sub>4</sub> hydrolase analogues that mimic at least a part of the three dimensional structure of the LTA<sub>4</sub> hydrolase molecule as defined by the parameters shown in Table 9 for atom 1 to atom 4876, which comprises the steps of
- (a) producing a multiplicity of analogue structures of LTA<sub>4</sub> hydrolase and
- 10 (b) selecting an analogue structure, wherein the three-dimensional configuration and spatial arrangement of one or more enzymatically active sites and/or binding sites of said LTA<sub>4</sub> hydrolase remain substantially preserved.
17. A method according to claim 16, wherein an analogue exhibiting an enzymatic activity, such as an epoxide hydrolase and/or aminopeptidase activity, is selected.
- Sub 125
- 15 18. A method according to claim 16 or 17, wherein an enzymatic inhibitor complementary to the amino acids defined in any one of claims 3, 4 or 5 is screened for.
19. An analogue obtainable by the method according to any one of claims 16-18.
20. An analogue according to claim 19, which exhibits an increased catalytic activity when compared to the naturally occurring form of LTA<sub>4</sub> hydrolase, such as defined in Table 9 by parameters of atom 1 to atom 4876.
- 20 21. A method for screening LTA<sub>4</sub> hydrolase binding compounds complementary to a region of LTA<sub>4</sub> hydrolase, preferably an enzymatically active site thereof, which comprises the steps of
- (a) producing a multiplicity of possible complementary structures and
- 25 (b) selecting a structure, wherein the three-dimensional configuration and spatial arrangement of regions involved in binding to LTA<sub>4</sub> hydrolase remain substantially preserved, which selection is based on the three-dimensional structure of LTA<sub>4</sub> hydrolase, and/or LTA<sub>4</sub> hydrolase complexed to an inhibitor thereof, in a form adopted thereof in nature, such as defined in Table 9.
22. A method according to claim 21, wherein a general metallohydrolase inhibitor is
- 30 selected, which is capable of inhibiting an enzyme belonging to the M1 family.

23. A method according to claim 21, wherein an inhibitor of the epoxide hydrolase activity and/or aminopeptidase activity of LTA<sub>4</sub> hydrolase or of LTC<sub>4</sub> synthases is selected.

24. A method according to claim 21, wherein a compound capable of antagonizing LTB<sub>4</sub> receptor binding of a cell is selected.

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Sub A6 } 25. A compound obtainable by the method according to any one of claims 21-24.

26. A method of engineering a protein, which method comprises the steps of  
-identification of a suitable set of mutations based on the structure of LTA<sub>4</sub> hydrolase;

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-generation of a library of genes which contains the suitable sequence variations;  
-selection of clones encoding the LTA<sub>4</sub> hydrolase analogues with a desired activity function;

wherein said desired activity is the capability of efficiently producing an organic compound of interest.

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27. A method according to claim 26, wherein the specified property is the suicidal mode of action of LTA<sub>4</sub> hydrolase.

Sub A7 } 28. A process for the purification of a protein according to any one of claims 1-3 or obtained according to claim 26 or 27, which purification includes hydroxyapatite-based chromatography and a subsequent anion exchange chromatography.

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29. A process for the crystallisation of an LTA<sub>4</sub> hydrolase, an analogue or a derivative thereof, wherein said crystallisation is performed with the addition of a ytterbium salt as an additive, such as an ytterbium chloride.

Sub A8 } 30. A protein obtained by the method according to any one of claims 27-29.

31. A protein according to claim 30, which is present in an essentially pure form.

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32. An isolated nucleic acid encoding a protein according to claim 30 or 31.

33. A nucleic acid capable of specifically hybridising to a the nucleic acid according to claim 32.

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34. Use of a protein, which is a genetically modified LTA<sub>4</sub> hydrolase, according to claim 30 or 31 in the preparation of LTB<sub>4</sub> or other metabolites in the leukotriene cascade.

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A9 } 35. A protein according to any one of claims 6-8, 25, 30 or 31 for use as a medicament.
- 5 36. Use of a protein according to any one of claims 6-8, 25, 30 or 31 in the manufacture of a medicament for the treatment and/or prevention of acute and chronic inflammatory and/or allergic disorders, said disorder being selected from the group consisting of arthritis, inflammatory bowel disease (IBD), psoriasis and chronic obstructive pulmonary disease (COPD); neoplasias and/or cancer; or disorders caused by the lethal factor of *Bacillus anthracis*, e.g. anthrax.
- 10 37. Use of a protein according to any one of claims 6-8, 25, 30 or 31, in the manufacture of a medicament for the treatment and/or prevention of an anti-inflammatory and/or anti-allergenic disorder, such as bronchial asthma, allergic rhinitis, conjunctivitis etc.
- 15 38. Use of a protein according to any one of claims 6-8, 25, 30 or 31 in the manufacture of a medicament for the treatment and/or prevention of infection caused by human immunodeficiency virus (HIV).